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HORNY LAYER LIPIDS

III. HYDROLYSIS STUDIES, BEFORE AND AFTER CHROMATOGRAPHY, OF THE POLAR (HEXANE INSOLUBLE) LIPIDS FROM STRATUM CORNEUM*

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The preceding paper (1) described studies of the overall composition of the horny layer lipids obtained from the prolonged extraction of stratum corneum, previously defatted with hexane, by means of very polar solvents. The horny layer lipids so obtained contain free fatty acids and neutral lipids as well as polar complex lipids which are insoluble in hexane. The free fatty acids and neutral lipids obviously represent breakdown products, formed during the extraction process, from more complex lipid substances and have not been studied in the present investigations.

The materials from which the horny layer lipids used in these studies were obtained are admittedly pathological. At the present time we are concerned with establishing the chemical structures of the complex polar lipids found in normal and pathological stratum corneum; once these have been elucidated we shall be better able to decide which lipids are derived by normal metabolism and which are the result of pathological processes.

EXPERIMENTAL

Materials and Methods

Scales. Large quantities of scales were obtained from a patient with an acute generalized exfoliative dermatitis of unknown origin, which subsequently resolved spontaneously. They were kindly made available to us by Dr. Peter Flesch (University of Pennsylvania). The scales, as received by us, were pulverized and had been extracted with hexane. They were thoroughly washed again with hexane before further extraction.

Solvents. All solvents were twice distilled through a 90 cm Vigreau column prior to use.

Extraction and isolation of the polar lipids. The extraction and fractionation procedure has been outlined in Fig. 1; the detailed description follows. The 'fat-free' scales were extracted, in batches of 10 to 50 g, with water (10 ml/g scales) by mechanical shaking for 16 hours. The water soluble compounds were isolated from the aqueous extract and weighed, while the residual scales (Residue 'A') were thoroughly dried. Residue 'A' was then extracted at room temperature with a mixture of chloroform and methanol (2:1; subsequently referred to as solvent C-M). The extraction was performed for a two week period during which the solvent was changed several times. At the end of this period the total extracts were collected together and the horny layer lipids recovered, after evaporation of the solvent, and weighed. The residual material, after extraction with solvent C-M (Residue 'B'), was weighed but not further studied.

The extracted horny layer lipids were suspended in hexane then filtered through a fritted-glass, pressure, filter-funnel. The precipitate was washed several times with hexane with thorough mixing after each solvent addition until a total volume of 500 ml hexane/g lipid had been used. The residue on the filter-funnel consisted of the polar lipids, these were then transferred with solvent C-M and weighed. The hexane extract was evaporated to dryness and the residue, consisting of free fatty acids and neutral lipids, weighed. The average results for the various batches processed by this method is shown in Table I. An average loss of 22% of horny layer lipids occurred in the hexane extraction process. This is believed to be caused by delipidation of certain proteolipids while in contact with the hexane (1).

Chemical determinations. Procedures, as described previously (1, 2), were used with the following additions. Phosphorus was determined by the method of King (3) and pentose by the method of McRary and Slattery (4).

Chromatography. Thin-layer chromatography (TLC) followed the previous description (5) with one modification. Solvent G (ether) has been replaced by several different mixtures of chloroform and methanol; thus solvent G 5 is chloroform containing 5% by volume methanol, while G 15 contains 15% methanol.

Column chromatography utilized silica exclusively; this was obtained from two sources. At first silicic acid (specially prepared for the chromatography of lipids), obtained from Bio-Rad Laboratories (Richmond, California), was used; later this was replaced by SilicAR CC-7 (Mallinckrodt Chemical Works, New York). The latter proved superior and gave columns with faster flow

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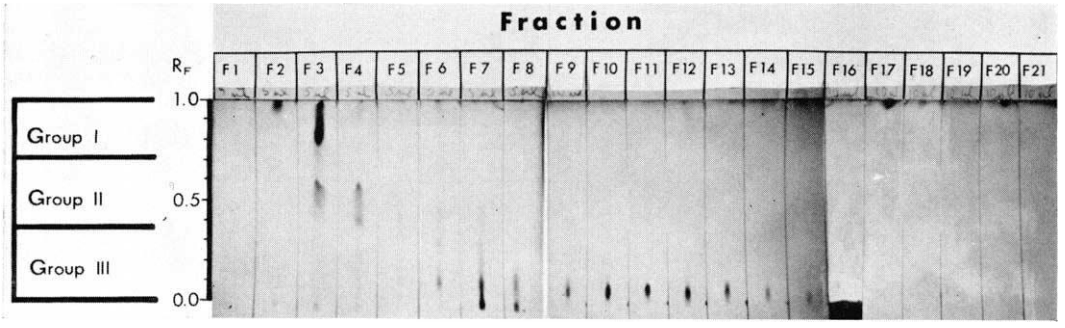

FIG. 1. Composite thin-layer chromatogram of the fractions isolated by primary chromatography (Exp. I).

rates. Before use both types of silica were washed thoroughly with solvent C-M, dried, and activated at 110° C for 1 hour. The columns used varied in diameter from 1 to 2.5 cm and in height from 23 to 90 cm. Elution commenced in most cases with pure chloroform, was continued with various chloroform-methanol mixtures (in subsequent sections 2% methanol would indicate a solvent containing 2% by volume methanol in chloroform, etc.), and was completed with pure methanol, as detailed in the later sections.

Ash determination. A sample of the polar lipids was ashed in a platinum crucible and the ash weighed. They were found to contain 3.2% ash.

Phosphorus determinations. Phosphorus determinations were performed, using the method of King (3), on samples of the original scales, the water soluble compounds, the polar lipids, together with two fractions subsequently isolated by chromatography. The results are shown in Table II.

1. Hydrolysis Studies before Chromatography

In all experiments 10 mg of the polar lipids were placed in a tube together with 1 ml of the appropriate solvent. The tube was sealed and then heated in a boiling water-bath for the prescribed period of time. After hydrolysis the tube was cooled and opened. The contents of the tube were extracted three times with 2 ml hexane (when alkaline hydrolysis had been performed the contents of the tube were acidified with HCl prior to this extraction). The combined hexane extract was washed with 1 ml water and the latter added to the aqueous phase. The washed hexane extract was evaporated to dryness and the residue of lipids weighed. The aqueous phase was similarly evaporated to dryness in a stream of nitrogen and the residue of non-lipids dried in vacuo and weighed. Sterols (sum of fast-acting and slow-acting) were determined, by the method of Moore and Baumann (6), on the extracted lipids; the latter were also studied by TLC (solvent D or S 1). Amino acids (7), hexosamine (8) and pentose (4) were determined on the residue from the aqueous phase.

TABLE I

Composition of 'fat-free' stratum corneum

Horny layer lipids		6.8%
Polar lipids (hexane insoluble)	2.8%	
Free fatty acid & neutral lipids	2.5%	
Water soluble compounds		13.7%
Residual 'keratinous' material		70.3%

TABLE II

The phosphorus content of various materials and isolated fractions

Substance	Phosphorus
	(%)
'Fat-free' scales	0.25
Water soluble compounds	0.75
Polar lipids (hexane insoluble)	0.56
Fraction F 47–50 (Exp. II)*	0.70
Fraction F 48 (Exp. III)*	1.60

* For explanation see text.

The results of these hydrolysis experiments are summarized in Table III. These show that after 8 hours hydrolysis with 2N HCl-methanol maximum amounts of lipids plus non-lipids are liberated and that these show little decline with further hydrolysis. In 16 hours liberation of amino acids is maximal and thereafter shows a steady decline. Liberation of sterols appears to be complete after 2 hours hydrolysis with 2N HCl-methanol or 3 hours hydrolysis with alcoholic KOH giving a percentage of 1.15%. A higher figure (2.5%) is obtained after 1 hour hydrolysis with N HCl (aqueous) but this is almost certainly due to formation of cholesta-diene. This would behave

TABLE III

Hydrolysis studies of the polar lipids before chromatography

Solvent Used	Time of Hydrolysis	Hydrolysis Product					
		Lipids	Non-lipids	Sterols	Amino Acids	Hexosamine	Pentose
	(*hr*)	(*g/100 g lipid*)					
2 N HCl in methanol	2	40.4	34.0	1.16	5.4	1.8	0.13
"	4	38.4	33.9	1.16	6.0	1.5	0.01
"	6	43.3	44.3	1.21	7.2	2.1	0.2
"	8	51.9	47.5	1.19	10.0	2.1	0.38
"	16	53.0	45.5	1.29	11.6	1.9	0.34
"	24	51.1	37.0	0.73	9.4	2.5	0.36
"	48	45.9	46.9	0.74	9.1	2.5	0.35
Aqueous 1 N HCl	5	78.6	14.0	2.5	2.0	1.1	—
N KOH in ethanol	3	53.4	—*	1.14	0.49	—	—
"	10	57.6	—*	0.86	7.3	—	0.25

* Owing to the large amounts of KCl produced on neutralizing it is not possible to obtain accurate values for the non-lipids liberated in these hydrolysis experiments which use alkaline conditions.

TABLE IV

Primary chromatography performed in Experiment I

Fraction	Eluant	Total volume (ml)	Weight eluted (mg)	As % original polar lipids
F 1	25% methanol*	7		
F 2–3	"	24.5	107.4	68.1
F 4–5	"	25	8.3	5.3
F 6–8	50% methanol	50		
F 9–11	75% methanol	45	16.4	10.4
F 12–23	Methanol	180		
F 24–31	"	120		

* For explanation see text.

as a fast-acting sterol and give anomalously high results.

2. *Chromatography Studies*

Experiment I

Primary chromatography. A sample of 157.5 mg of the polar horny layer lipids was dissolved in a small volume of chloroform with added methanol, loaded onto a 1 x 25 cm column of silicic acid Bio-Rad), and chromatographed with chloroform-methanol mixtures. Details are shown in Table IV. The eluates were collected in volumes of about 15 ml, each evaporated to dryness and the residue examined by TLC (solvent H). A composite photograph of the TLC results of fractions F 1–21 is shown in Fig. 2; classification into the three groups follows our previous convention (1). Most of the material was eluted in the first eight fractions with little separation; subsequent fractions showed a major component with traces of other components. Fractions F 17 and F 20 show the presence of components travelling close to the solvent front; it is concluded that these represent breakdown products formed by the action of methanol on the highly polar 'proteolipids' still remaining on the column. Recovery from the column was only 78.6%; this is further suggestive evidence that breakdown occurred during chromatography with the liberation of non-lipid material which was not eluted from the column.

Preparative TLC. An attempt was made to utilize preparative TLC for further resolution of the crude fractions obtained by primary chromatography. Fractions F 4 and F 5 were pooled (weight 8.5 mg) and submitted to preparative chromatography TLC (0.25 mm silica; solvent H). After chromatography the plate was cut into three zones and each zone eluted with solvent C-M. As in previous studies (1) good resolution was obtained with this method but we were not successful in devising a procedure to remove the heavy contaminant of silica. No further studies were made with preparative TLC.

Secondary chromatography. In an attempt to obtain further resolution fractions F 2 and F 3, from the primary chromatography, were pooled (weight 107.4 mg) and rechromatographed on a 2.5 x 23 cm column of silicic acid (Bio-Rad). Details are shown in Table V; all eluted fractions were examined by TLC (solvent H). A more gradual stepwise gradient with methanol was used in this experiment and somewhat better separation was achieved as shown by the results of the TLC studies (Table VI). Further evidence of the breakdown of the polar components is indicated though recovery is better (93%).

Studies of fraction R 14. This fraction (15.2 mg) was crystallized from solvent C-M to give 12.2 mg of pale yellow crystals, m.p. 102–5° C (uncorr.). Examination by TLC (solvent H) indicated a single component (R_F 0.95) with trace contamination. Saponification with N KOH liberated 73% of lipids (hexane extract) and 16.4% free amino acids. Studies of the liberated lipids by TLC (solvent S1) indicated the presence of some fatty acids, some monohydric alcohols and some polar material which was either hydroxylated fatty acids or dihydric alcohols.

TABLE V

Secondary chromatography performed in Experiment I

Fraction	Eluant	Total volume (ml)	Weight eluted (mg)	As % original polar lipids
R 1–8	Chloroform	105	—	—
R 9–11	5% methanol*	45	—	—
R 12	"	15	0.4	0.3
R 13	"	15	45.6	28.9
R 14	"	15	15.2	9.6
R 15	"	15	5.0	3.2
R 16	"	15	6.1	3.9
R 17	"	15	8.3	5.3
R 18	"	15	2.0	1.3
R 19–22	"	60	—	—
R 23–31	10% methanol	135	—	—
R 32–41	25% methanol	150	—	—
R 42–49	50% methanol	120	—	—

* For explanation see text.

TABLE VI

TLC studies of fractions obtained in secondary chromatography in Experiment I

Fraction	
R 1–8	Group I* with a trace of Group III
R 9–12	Group I only
R 13–15	Group I with trace of Group II
R 16–22	Groups I and II with traces of Group III
R 23–25	Groups I and II
R 26–31	Group II with some Groups I and III
R 32–38	Mainly Group II with traces of Groups I and III; Fraction R 37 gives a blue intermediate color with H_2SO_4
R 39–41	Negligible traces only
R 42–49	Traces of Groups I and III

* See Fig. 2 and text for explanation of Groups.

TABLE VII

Primary chromatography performed in Experiment II

Fraction	Eluant	Total volume (ml)	Weight eluted (mg)	As % original polar lipids
F 1	20% methanol	75	—	—
F 2	"	15	1319.7	69.7
F 3	"	25	506.8*	26.8
F 4	"	25	74.6*	3.9
F 5–8	"	225	—	—
F 9–23	Methanol	350	92.7*	4.9

* Silica present in these specimens.

TABLE VIII

Secondary chromatography of fraction 2M of Experiment II

Fraction	Eluant	Total volume (ml)	Weight eluted (mg)	As % original polar lipids
R 1–22	Chloroform	356.5	306.4	16.2
R 23–25	2% methanol	45	*	—
R 26–38	5% methanol	295	*	—
R 39–46	10% methanol	200	*	—
R 47–55	Methanol	320	*	—

* Weights not significant due to leak in column.

Experiment II

Primary chromatography. A sample of 1892.2 mg of the polar horny layer lipids was dissolved in minimum volume of 20% methanol in chloroform and loaded onto a 2 x 26 cm column of silicic acid (Bio-Rad). Elution was commenced with 20% methanol and a pressure of 5–8 psi N_2 applied to maintain an adequate flow. Elution was completed with methanol; details of the chromatogram are shown in Table VII. Most of the material eluted in the first four fractions without resolution.

Studies of fraction F 2. This fraction (1319.7 mg) was crystallized from chloroform to give 387.4 mg. of light yellow amorphous powder with an indefinite melting point (85–95° C; uncorr.). Thin-layer chromatography (solvent H) indicated that no significant purification had resulted from the recrystallization. On evaporation of the mother liquors 823.9 mg of material was recovered. This material was readily soluble in chloroform and will subsequently be referred to as fraction F 2M.

Secondary chromatography. In an attempt to resolve fraction F 2M a long column (1 x 90 cm) of silicic acid (Bio-Rad) was used. The column tube consisted of two 50 cm segments joined by a Viton 'O'-ring connection and hence less of the material chromatographed. The sample was loaded

TABLE IX

Tertiary chromatography performed in Experiment II

Chromatogram	Secondary chromatography fractions pooled	Eluants	Eluants containing major peak
A	R 3–15	(a) Chloroform (10)*	
		(b) 10% methanol (5)	A 4–7
		(c) methanol (1)	
B	R 16–22	Chloroform (20)	B 8–13
C	R 23–31	(a) Chloroform (8)	
		(b) 5% methanol (8)	C 4–9
		(c) Methanol (1)	
D	R 32–39	(a) 5% methanol (9)	
		(b) 10% methanol (3)	D 8–11
		(c) Methanol (1)	
E	R 40–54	(a) 10% methanol (8)	E 3–5
		(b) 50% methanol (4)	E 10–12
		(c) Methanol (2)	
F —	R 55	(a) 5% methanol (5)	
		(b) 50% methanol (9)	F 8–17
		(c) Methanol	

* Figures in parenthesis indicate number of fractions (5–10 ml) of each eluant collected.

onto the column with chloroform and chromatography commenced with this solvent. Details of this chromatography are shown in Table VIII. As in preceding experiments each eluted fraction was examined by TLC (solvent H): the results showed that better resolution had been obtained under these conditions of chromatography. Fractions R 1–39 contained Group I compounds almost exclusively, though fractions R 11–15 contained a Group II compound which did not give the intermediate coloration characteristic of sterols. Fractions R 40–51 contained the sterol-positive Group II compounds with varying amounts of Group I compounds. Only traces of Group III compounds were present in any of these fractions.

Tertiary chromatography. Further purification of the fractions obtained in the secondary chromatography was attempted by rechromatography. The fractions were pooled in seven groups and chromatographed separately on 1 x 25 cm columns of silicic acid (Bio-Rad). Details are tabulated in Table IX.

Examination by TLC (solvent G 15) of fractions obtained in chromatogram A showed the presence of two components (R_F 0.95 and 0.85) which failed to separate. Chromatogram B, when examined by TLC (solvent H), showed a single component (R_F 0.95); this material was recrystallized. Examination of the fractions from chromatogram C by TLC (solvent H) showed the presence of Group I compounds only; fraction C 8 gave an intermediate blue spot with H_2SO_4 (R_F 0.85). More careful TLC, with solvent G 15, indicated that there were two groups of compounds, one with R_F above 0.7 and the other with an R_F of 0.1. These two groups were not separated by the rechromatography, neither did recrystallization resolve them. Similar examination of the fractions from chromatogram D (TLC; solvent G 15) showed the presence of a single component R_F 0.7; while chromatogram E showed two groups of compounds (TLC; solvent G 15) with R_F 0.5–0.6 and 0.1–0.2 respectively. In chromatogram F compounds with hgh R_F (TLC; solvents G 15 or H) were removed in the first three fractions; subsequent fractons showed the presence of compounds wth R_F 0.0–0.5 only.

Experiment III

Primary chromatography. A sample of 1298.5 mg of the polar lipids was chromatographed on a 2 x 35 cm column of SilicAR CC-7 (Mallinckrodt) as shown in Table X; a pressure of 5 psi N_2 was ap-

TABLE X

Primary chromatography performed in Experiment III

Fraction	Eluant	Total volume (ml)	Weight eluted (mg)	As % original polar lipids
F 1–5	Chloroform	100	3.0	0.2
F 6–7	"	27.5	306.5	23.6
F 8–9	2% methanol	80	215.8	16.6
F 10–16	"	175	343.5	26.5
F 17–28	10% methanol	345	62.3	4.8
F 29	"	20	107.0	8.2
F 30–34	20% methanol	131.5	38.8	3.0
F 35–38	"	100	11.5	0.9
F 38–46	"	205	9.6	0.7
F 47	Methanol	75	4.6	0.4
F 48	"	200	29.7	2.3
F 49	"	100	8.3	0.6
F 50	"	100	—	—

plied to the column during chromatography. This brand of silica was superior, a more gradual stepwise gradient of methanol used, and improved separation obtained as shown by TLC examination of the eluted fractions. Recovery from the column was 87.8%.

Secondary chromatography. Fractions F 29–46 from the primary chromatography contained the sterol-positive Group II compounds. These fractions were pooled together and rechromatographed on a 1 x 25 cm column of the SilicAR CC-7. Examination by TLC (solvent G 15) showed that the sterol-containing compounds were present in fractions R 17–21. Details of the rechromatography are shown in Table XI.

TABLE XI

Secondary chromatography performed in Experiment III

Fraction	Eluant	Total volume (ml)	Weight eluted (mg)	As % original polar lipids
R 1–7	Chloroform	80	—	—
R 8–15	1% methanol	80	—	—
R 16	10% methanol	10	58.3	4.6
R 17–21	10% methanol	56	51.3	4.0
R 22–34	33% methanol	122.5	—	—
R 35–6	Methanol	130	—	—

3. *Hydrolysis Studies of the Chromatographic Fractions from Experiments II and III*

Fractions, either singly or pooled, from the two preceding experiments were subjected to hydrolysis using the technique described in section 1 (hydrolysis studies before chromatography). Hydrolysis was performed in both acid and alkaline conditions on each specimen with the results shown in Table XII.

These studies permit the following general conclusions. The group I compounds contain approximately 70% lipids and 8% amino acids; no phosphorus and only traces of hexosamines are present; free fatty acids and sterols were detected in the products of hydrolysis. The major Group II compound appears to contain about 8% sterol and 46% of other lipids; further examination is described below. The Group III compounds appear to contain 32% lipids, 67% amino acids and 1–2% phosphorus; further examination is described below.

Comparison of the results obtained from hydrolysis before chromatography with those obtained on isolated fractions shows some significant features. We have been able to account for all the sterol present in the original polar lipid material in the fractions isolated, while a loss of about 20% of the amino acids occurs. On the other hand we appear to have lost almost all of the hexosamine.

TABLE XII

Hydrolysis studies performed in fractions isolated by chromatography

Fraction*	Conditions of Hydrolysis	Hydrolysis Products					
		Lipids	Non-lipids	Sterols	Amino Acids	Hexosamine	Pentose
		(*g/100 g lipid*)					
F 6-7 (Exp. III)	5 hr. with N HCl (aq)	78.3	8.5	0.69	0.65	0.10	0.0
"	3 hr. with N KOH (alc)	69.4	—†	0.16	8.7	0.25	0.13
F 8–9 (Exp. III)	5 hr. with N HCl (aq)	89.9	0.7	2.9	0.7	0.06	1.7
"	3 hr. with N KOH (alc)	52.6	—†	0.58	6.5	0.06	—
F 10–15 (Exp. III)	5 hr. with N HCl (aq)	—	26.2	3.4	1.1	0.09	2.8
"	3 hr. with N KOH (alc)	48.0	—†	0.14	5.5	0.17	0.0
F 16 (Exp. III)	4 hr. with N HCl (aq)	80.0	20.0	—	—	0.33	0.2
"	3 hr. with N KOH (alc)	60.7	—†	4.6	—	—	—
F 17–21 (Exp. III)	4 hr. with 2 N HCl (Meth)	53.6	46.2	7.3	—	—	—
"	4 hr. with N HCl (aq)	71.3	29.4	—	—	0.17	—
"	2 hr. with KOH (alc)	39.6	—†	2.9	0.13	—	—
F 17–28 (Exp. III)	5 hr. with N HCl (aq)	79.0	12.9	0.78	0.60	1.25	0.0
"	3 hr. with N KOH (alc)	10.3	—†	0.65	4.4	0.75	0.18
F 47–50 (Exp. II)	5 hr. with N HCl (aq)	4.5	30.9	0.16	27.7	0.25	0.0
"	3 hr. with N KOH (alc)	32.3	—†	0.22	66.5	0.25	0.75
"	5 hr. with N KOH (alc)	31.9	—†	0.15	68.5	0.88	0.85

* A range of numbers indicates that the corresponding fractions have been pooled.

† Contains KCl (of footnote to Table III).

Prior to chromatography the material contained 2–2.5% hexosamine yet in most of the specimens isolated after chromatography negligible amounts of hexosamine is present. Fraction F 17–28 contains only 1.25% and this fraction is only 4.8% of the original material. More than 90% of the hexosamine originally present has been lost during chromatography. We can offer no explanation for this loss. In present studies we have determined hexosamine and pentose only; studies of other carbohydrates are in progress.

The preceding represents our general conclusions; specific comments on three fractions which were subjected to a rather more detailed study follows.

Fraction R 17–21 (Experiment III). Thin-layer chromatography of the lipids isolated after hydrolysis of this fraction showed the presence of sterols, the major component of which appeared to be cholesterol, and fatty acids. Some polar material remained at the origin (solvent S 1) which may have been hydroxy-fatty acids. Amongst the other products of hydrolysis we were able to demonstrate the presence of appreciable amounts of uracil. The identity of the latter was confirmed by paper chromatography followed by ultraviolet spectroscopy of the eluted fraction. The ultraviolet absorption curve was characteristic for uracil (λ_{max} 259 mμ).

Fraction F 47–50 (Experiment II). This material contains large amounts of amino acids (67%) and much less lipid material than did the preceding fractions. At least eight amino acids are present, the predominant one being valine; further studies are in progress. All the phosphatides present in the polar lipids appear concentrated in this fraction; the major phosphatide has been identified as sphingomyelin and further studies are being made.

Fraction F 48 (Experiment III). Careful recrystallization of this fraction gave bright yellow leaflets which melt sharply with decomposition, m.p. 150° C (uncorr.).

DISCUSSION AND CONCLUSIONS

The major objective of this study is to develop methods for handling, separation and analysis of the polar lipids from human stratum corneum. Previous studies (1) have indicated that these compounds are of unknown chemical nature and hence appropriate analytical techniques must be developed for their study. Primary chromatography, on silica columns, followed by repeated rechromatography appears to be the only method available at present for the separation of these compounds. This method has serious limitations since we have repeatedly found evidence indicating breakdown of more complex compounds during the chromatographic procedures. Such breakdown appears to be the result of solvent action rather than an effect of the column material since we have observed similar breakdown of solutions kept at refrigeration temperatures. Consequently, as polar solvents are required both for the extraction of the lipids from the stratum corneum and for any type of chromatographic separation, we can see no way of avoiding this continual breakdown during the analytical processes.

In spite of these difficulties we have, nevertheless, been able to isolate several substances in a pure, and sometimes crystalline, form. While we would hesitate to claim that these are the original complex lipids of stratum corneum we believe that they represent fragments produced by breakdown of the original complexes. If we are correct in our conclusion that they are breakdown fragments rather than artifacts or oxidation products then the elucidation of their chemical structure is relevant.

A detailed study has been made of the mixed polar lipids and of isolated fractions. We have found it necessary to perform hydrolysis under both acid and alkaline conditions to obtain the required data. The identification of hydrolysis products is often incomplete; neither have we been able to elucidate the complete structure of any one compound. Nevertheless the results of these studies support, in general details, previous findings (1) and in addition we have obtained more detailed information concerning the chemical nature and behaviour of these complex lipids. New findings include the following:

The Group I polar lipids comprise some two thirds of the total polar lipids studied in this particular investigation. The amino acid content of this material is low (less than 10%) and hence this fraction probably consists of lipo-amino acids together with unidentified lipids which contain neither amino acid nor phosphorus. The latter probably represent the lipid fragments from the original lipid-protein complexes of the stratum corneum. We are not justified in using the term proteolipids for this group of compounds.

The Group II polar lipids contain a sterol-positive substance, as observed previously (1). We have continued our study of this group of compounds and have isolated the sterol-material in an apparently pure form. The fraction accounts for only about 2% of the horny layer lipids used in this study. The

material we have isolated has been found to contain appreciable amounts of uracil, but the sterol content is much lower than we expected (7.3%) and negligible amounts of hexosamine are present. This fraction does not appear to be identical with the corresponding sterol-compound isolated previously (1) from psoriatic scales. There thus appear to be present in stratum corneum, both normal and pathological, a number of sterol-containing polar lipids of varied and unknown structure. In this context we would like to repeat our previous suggestions (1) that these polar sterol-compounds play a role in the pathology of the scaling dermatoses. The fact that the compounds are entirely different in two different skin diseases is indeed significant. This group of compounds contains neither amino acids nor phosphorus. The term proteolipid is irrelevant for this group.

We have been successful in isolating one of the Group III compounds in a crystalline form. Our analytical data suggests that this group of compounds contain members which are phosphatide-peptide complexes. While our data are inadequate to propose even a tentative structure we can postulate as one possible compound a sphingomyelin complex containing a peptide chain of some fifteen amino acids joined by the carboxyl end-group to the free hydroxyl group of the sphingosine. We are continuing our study of these compounds. While the term proteolipid could be applied to the Group III compounds we consider that lipopeptide would be more correct.

SUMMARY

1. Further studies have been made of the chemical nature of the lipids from human stratum corneum.

2. Methods have been devised for the handling, separation and analysis of these compounds. While these methods have serious limitations we have, nevertheless, been able to isolate certain compounds in crystalline form.

3. Polar sterol containing substances have been isolated from shed scales of a patient with an exfoliative dermatitis. This sterol-material is not identical with similar material isolated, in a previous study, from psoriatic scales.

4. Some progress has been made in the elucidation of the nature of the more polar (Group III) compounds and a tentative suggestion has been made that these are phosphatide-peptide complexes.

5. While true proteolipids may be present in the unextracted horny layer materials the compounds isolated so far cannot be so classified. The majority appear to be very polar complex lipids of unknown structure. Some could correctly be called lipo-peptides. The original proteolipids of stratum corneum appear to break down during extraction and other analytical processes, and only breakdown products of varying complexity are obtained for study.

REFERENCES

1. Wheatley, V. R. and Flesch, P.: Horny layer lipids. II. Further studies on the overall chemical composition of lipids from normal and pathological human stratum corneum. J. Invest. Derm., *49:* 198, 1967.
2. Wheatley, V. R., Flesch, P., Esoda, E. C. J., Coon, W. M. and Mandol, L.: Studies of the chemical composition of the horny layer lipids. J. Invest. Derm., *43:* 395, 1964.
3. King, E. J.: The colorimetric determination of phosphorus. Biochem. J., *26:* 292, 1932.
4. McRary, W. L. and Slattery, M. C.: The colorimetric determination of pentoses and pentosans. Arch. Biochem., *6:* 151, 1945.
5. Wheatley, V. R.: Cutaneous lipids. III. An integrated system for the analysis of cutaneous lipids. Proc. Sci. Section TGA, *42:* 43, 1964.
6. Moore, P. R. and Baumann, C. A.: Skin sterols. I. Colorimetric determination of cholesterol and other sterols in skin. J. Biol. Chem., *195:* 615, 1952.
7. Harding, V. J. and MacLean, R. M.: A colorimetric method for the estimation of amino nitrogen. J. Biol. Chem., *24:* 503, 1916.
8. Elson, L. A. and Morgan, W. T. J.: A colorimetric method for the determination of glucosamine and chondrosamine. Biochem. J., *27:* 1824, 1933.